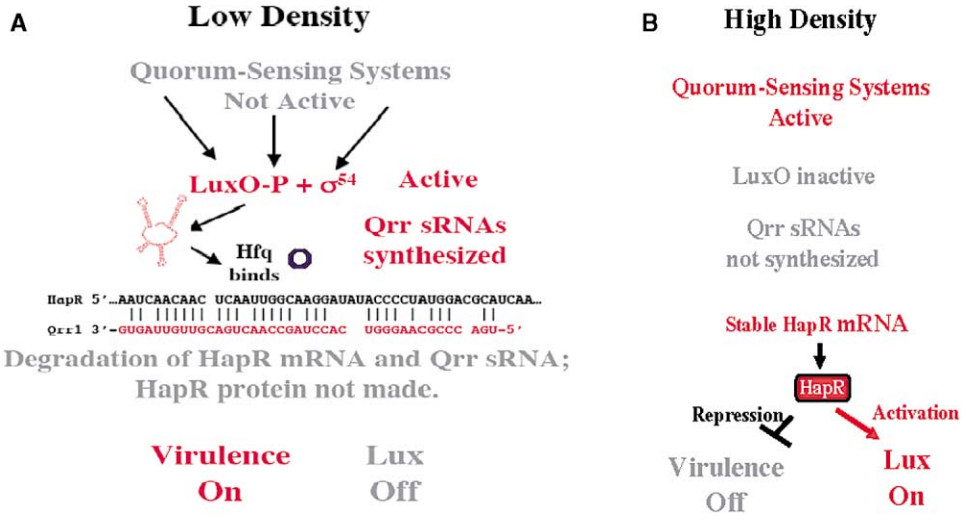

Figure 1. Low Density Signaling for Qrr RNA Synthesis

(A) In the absence of quorum sensing (low density), LuxO is phosphorylated. The phosphorylated form is a positive regulator of Qrr RNA synthesis; the Qrr RNAs negatively regulate HapR mRNA stability and translation.

(B) At high density, LuxO is not phosphorylated, and the Qrr RNAs are not made. See Lenz et al. (2004) for more details.

tion, act on the same region of the same target message (reviewed in Repoila et al. [2003]). In *Pseudomonas aeruginosa*, two redundant small RNAs with subtly different induction conditions regulate iron homeostasis (Wilderman et al., 2004). sRNAs with varying degrees of redundancy may turn out to be a common cell strategy for precise regulation.

The appearance of four small RNAs in the center of the signaling cascade for quorum sensing and virulence is undoubtedly a sign of things to come. In fact, a regulatory RNA, RNAIII, has been known for years to play a critical role in *S. aureus* virulence (reviewed in Johansson and Cossart [2003]). Watch for small RNAs in all your favorite regulatory circuits.

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Selected Reading

Bartel, D.P. (2004). Cell *116*, 281–297.

Gottesman, S. (2004). Annu. Rev. Microbiol. *58*, 303–328. Published online June 16, 2004. 10.1146/annurev.micro.58.030603.123841.

Johansson, J., and Cossart, P. (2003). Trends Microbiol. *11*, 280–285.

Lenz, D.H., Mok, K.C., Lilley, B.N., Kulkarni, R.V., Wingreen, N.S., and Bassler, B.L. (2004). Cell *118*, this issue, 69–82.

Lilley, B.N., and Bassler, B.L. (2000). Mol. Microbiol. *36*, 940–954.

Massé, E., and Gottesman, S. (2002). Proc. Natl. Acad. Sci. USA *99*, 4620–4625.

Massé, E., Escorcia, F.E., and Gottesman, S. (2003). Genes Dev. *17*, 2374–2383.

Miller, M.B., and Bassler, B.L. (2001). Annu. Rev. Microbiol. *55*, 165–199.

Repoila, F., Majdalani, N., and Gottesman, S. (2003). Mol. Microbiol. *48*, 855–861.

Storz, G., Opdyke, J.A., and Zhang, A. (2004). Curr. Opin. Microbiol. *7*, 140–144.

Wilderman, P.J., Sowa, N.A., FitzGerald, D.J., FitzGerald, P.C., Gottesman, S., Ochsner, U.A., and Vasil, M.L. (2004). Proc. Natl. Acad. Sci. USA *101*, 9792–9797.

Neddylating the Guardian: Mdm2 Catalyzed Conjugation of Nedd8 to p53

The tumor suppressor and transcriptional regulator p53 is perhaps one of the most regulated proteins in the cell nucleus and is acted upon by a variety of protein kinases, acetylases, ubiqutin ligases and hydrolases, and SUMO-conjugating enzymes. Now new work suggests a role for an additional modification—neddylation—in negative regulation of p53 transcriptional activity.

The tumor suppressor p53 is a central component in the signal transduction pathway that responds to cellular and genotoxic stress. p53, through its transcription factor activity, induces the expression of genes involved in DNA repair, cell cycle arrest, and apoptosis. The key position that p53 takes in the stress pathway dictates that its activity be highly regulated, and multiple mechanisms, including phosphorylation, acetylation, and ubiquitination-mediated degradation, conspire to regulate its activity (Yang et al., 2004). To the casual observer, the status of our understanding of p53 and its regulation is likely to present a paradox. On one hand, p53 is already one of the most intensively studied proteins in

eukaryotes (Sherr, 2004). On the other hand, we are constantly bombarded with new mechanisms that control p53 function, and with each new discovery, we are reminded of how little is actually understood. Although the involvement of the Mdm2 ubiquitin ligase in p53 turnover has been known for almost a decade, recent studies have revealed a varied assortment of pathways that impinge on p53 turnover, including COP1, Pirh2, YY1, and p300-mediated ubiquitination, PARC-mediated sequestration in the cytoplasm, reversal of ubiquitination by deubiquitinating enzymes, and sumoylation (reviewed in Yang et al. [2004]) (Figure 1C). However, we know extremely little about how these pathways interact in a dynamic sense. Thus, a common response may be to throw one's hands up in dismay at the complexity of it all. Now work from the laboratory of David Lane (Xirodimas et al., 2004 [this issue of *Cell*]) adds yet another modification to the mix, neddylation.

Neddylation is the process by which the C-terminal glycine of the ubiquitin-like protein Nedd8 is covalently linked to lysine residues in a protein through an isopeptide bond, analogous to protein ubiquitination (reviewed in Cope and Deshaies [2003]). However, unlike ubiquitination, Nedd8 does not generate polymeric chains. However, Nedd8 is required for protein degradation through its activator function for SCF (Skp1/Cul1/F-box protein) ubiquitin ligases (Figure 1A). The core component of SCF complexes is the cullin subunit. All known cullins are conjugated to Nedd8 on a single lysine residue located in the C-terminal domain that assembles with the Rbx1 RING-H2 finger protein (Zheng et al., 2002). This modification requires the Nedd8-specific Ubc12, a two-component Nedd8-activating enzyme (NAE1), and the Rbx1 RING finger (Cope and Deshaies, 2003). Once cullin is neddylated, its ability to promote ubiquitination is greatly enhanced (Read et al., 2000; Wu et al., 2000). This modification is removed by a metalloprotease activity in the COP9/signalosome complex (Cope and Deshaies, 2003).

Until recently, only cullins have been detected in conjugation with Nedd8 (Stickle et al., 2004). Thus, it is with some surprise that Xirodimas et al. (2004) now report that Nedd8 can be conjugated to p53 and its regulator Mdm2. The fact that MDM2 is a RING finger E3 that has autoubiquitination activity led Xirodimas et al. to ask whether Nedd8 can be conjugated to Mdm2 in $p53^{-/-}$ tissue culture cells. While Mdm2 was found to undergo conjugation with Nedd8 in transfected cells, Mdm2 RING finger mutants did not. Because Mdm2 can conjugate ubiquitin to p53 (reviewed in Yang et al. [2004]), Xirodimas et al. examined whether p53 could also be neddylated and, if so, whether Mdm2 was involved. When both Nedd8 and p53 were overexpressed, small amounts of Nedd8 were found conjugated to p53. However, in the presence of Mdm2, the amount of Nedd8-conjugated p53 was dramatically increased (Figure 1B). This modification was reversed by coexpression of the Nedd8-specific protease (NEDP1) but not by the SUMO-specific protease SSP3. The ability of Mdm2 to promote neddylation of p53 was specific in that the conjugation of other ubiquitin-like proteins—SUMO and ISG15—was not stimulated by Mdm2 and also requires phenylalanine-19 in p53, a residue known to be required for interaction with Mdm2. In vitro, Ubc12 and NAE1 were sufficient to conjugate small amounts of Nedd8 to p53; however, this reaction was only modestly stimulated by Mdm2 while p53 ubiquitination was dramatically stimultated (Xirodimas et al., 2004). It is unclear at this point whether efficient conjugation of Nedd8 to p53 requires other components, such as YY1, which functions with Mdm2 in p53 ubiquitination (Sui et al., 2004).

A central question was whether endogenous p53 and Mdm2 are neddylated in intact cells. In reciprocal coimmunoprecipitation experiments, endogenous p53 and Mdm2 were found to be covalently associated with Nedd8. One limitation in the current study is the absence of a quantitative assessment of the fraction of p53 that is modified and whether this modification is regulated during the cell cycle. In typical immunoblot experiments of cell lysates, neddylated p53 is not detected, suggesting that only small amounts of p53 are neddylated at steady state. However, the finding that depletion of Mdm2 by RNAi reduces the steady state amount of p53 neddylation indicates a supportive role for Mdm2 in this modification in vivo.

While ubiquitination is known to occur on as many as six lysine residues in p53, mutagenesis studies revealed that only three of these are required for efficient neddylation (Xirodimas et al., 2004). Thus, p53 molecules can be both ubiquitinated and neddylated simultaneously. Whether there is an order to the conjugation process in vivo is not clear, but it is conceivable that Mdm2 can employ both conjugating systems during a single engagement of p53. It is unclear at present whether p53 that is both neddylated and ubiquitinated is an efficient substrate for the proteasome. To generate in vivo evidence of a role for neddylation in controlling p53 activity, the authors turned to a neddylation-deficient p53 mutant and the ts-41 cell line, which contains a temperature-sensitive subunit of the NAE1 complex (APP-BP1). The transcriptional activity of the neddylation-deficient mutant of p53 was higher than wild-type p53 at the permissive temperature and did not significantly increase further after shift to the restrictive temperature, suggesting an inhibitory role for p53 neddylation (Figure 1B). These functional experiments employed transient expression systems together with plasmid reporter constructs. The question of whether p53 neddylation by Mdm2 normally contributes to inhibition of transcriptional activity toward endogenous target genes remains unanswered. Of particular interest is whether neddylation of a small fraction of p53 leads to a reduction in transcription at multiple p53-responsive promoters or whether only a subset of p53-regulated genes are affected upon p53 neddylation.

A central role for p53 is activation of the transcriptional branch of the DNA damage checkpoint. In this process, p53 is phosphorylated by multiple kinases, and these signals impair Mdm2-dependent p53 turnover. Thus, a central question is whether p53 neddylation is regulated in response to DNA damage. Xirodimas et al. examined the effect of ultraviolet radiation (UV) on p53 modification. While UV dramatically decreased in the extent of p53 polyubiquitination, the levels of neddylated p53 were increased at early times after UV treatment. This could reflect regulation of p53 neddylation during the

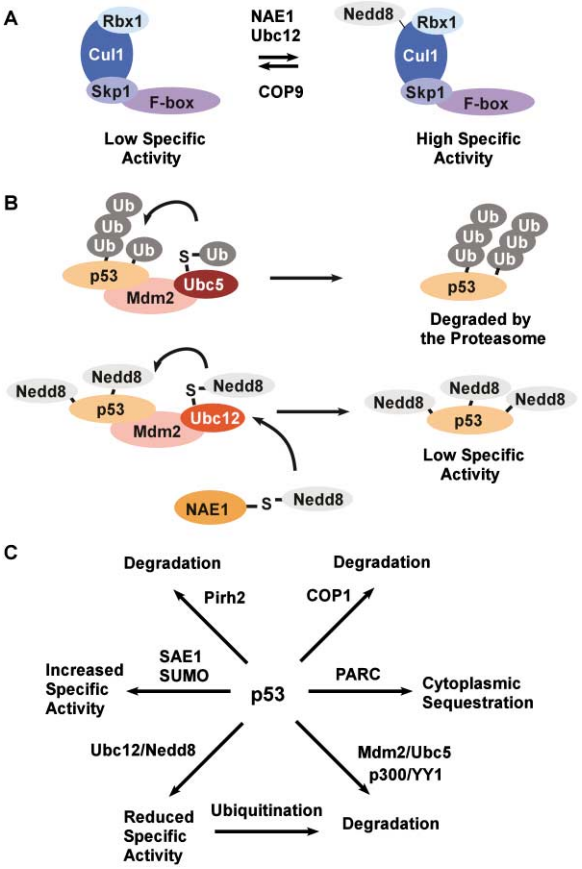

Figure 1. Nedd8 Conjugation Pathways

(A) Nedd8 is covalently attached to members of the cullin family including Cul1 via the activity of NAE1 and Ubc12. COP9 is responsible for removing Nedd8 from cullins.

(B) The C-terminal RING domain of Mdm2 is used to promote neddylation of at least three lysine residues in p53, which interacts with the N terminus of Mdm2. Neddylation appears to reduce the transactivation activity of p53. In contrast, ubiquitination occurs through recruitment of conjugating enzymes such as Ubc5 to the RING domain of Mdm2, leading to degradation of p53.

(C) Multiple pathways impinge on p53 modification by ubiquitin-like proteins.

DNA damage response or could simply be a consequence of increased p53 levels. Unlike ubiquitination, DNA damage-dependent phosphorylation does not appear to block neddylation. This raises the issue of whether Mdm2 is responsible for p53 neddylation after damage. DNA damage-dependent phosphorylation blocks association with Mdm2 with p53 (Yang et al., 2004) and, based on mutagenesis experiments (Xirodimas et al., 2004), would presumably block neddylation as well. An additional question concerns removal of Nedd8. In SCF complexes, Nedd8 is removed by the action of the COP9 complex (Cope and Deshaies, 2003). How and under what circumstances Nedd8 is removed from p53 is unknown, although at long times after DNA damage, the levels of neddylated p53 were reduced while total p53 levels remained high, suggesting the existence of a deneddylation pathway. However, given the fact that neddylated p53 can also be ubiquitinated, it is possible that neddylated p53 may also undergo proteasome-mediated degradation in certain situations, negating a need for regulated deneddylation. Clearly, understanding the dynamic relationship between p53 neddylation, p53 ubiquitination, and p53 turnover will be required to determine how neddylation fits into the p53 degradation pathway.

As is often the case with novel findings, this work raises as many questions as it answers. In particular, it is not clear under what physiological settings p53 neddylation occurs, nor is it evident precisely how neddylation regulates p53 function. Does neddylation play a critical role overall in p53 biology or is it a minor component of p53's diverse regulatory apparatus? In addition, this work raises the question of whether Nedd8 transfer through RING-based E3s is a frequent event. If Mdm2 is a harbinger of things to come, then there could be many more RING-based E3s that are capable of functioning together with Ubc12 to promote neddylation of proteins that are otherwise targets of a RING-based ubiquitin ligase activity. If this is the case, then an intermingling of neddylation and ubiquitination could be the rule and not the exception.

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Selected Reading

Cope, G.A., and Deshaies, R.J. (2003). Cell *114*, 663–671.

Read, M.A., Brownell, J.E., Gladysheva, T.B., Hottelet, M., Parent, L.A., Coggins, M.B., Pierce, J.W., Podust, V.N., Luo, R.S., Chau, V., and Palombella, V.J. (2000). Mol. Cell. Biol. *20*, 2326–2333.

Sherr, C.J. (2004). Cell *116*, 235–246.

Stickle, N.H., Chung, J., Klco, J.M., Hill, R.P., Kaelin, W.G., Jr., and Ohh, M. (2004). Mol. Cell. Biol. *24*, 3251–3261.

Sui, G., Affar, E.B., Shi, Y., Brignone, C., Wall, N.R., Yin, P., Donohoe, M., Luke, M.P., Calvo, D., Grossman, S.R., and Shi, Y. (2004). Cell *117*, 859–872.

Wu, K., Chen, A., and Pan, Z.Q. (2000). J. Biol. Chem. *275*, 32317–32324.

Xirodimas, D.P., Saville, M.K., Bourdon, J.-C., Hay, R.T., and Lane, D.P. (2004). Cell *118*, this issue, 83–97.

Yang, Y., Li, C.-C.H., and Weissman, A.M. (2004). Oncogene *23*, 2096–2106.

Zheng, N., Schulman, B.A., Song, L., Miller, J.J., Jeffrey, P.D., Wang, P., Chu, C., Koepp, D.M., Elledge, S.J., Pagano, M., et al. (2002). Nature *416*, 703–709.

Huntington's Disease: New Paths to Pathogenesis

Huntington's disease is a progressive autosomal dominant neurodegenerative disorder caused by expan-